



BOX PATENT

Attorney Docket No. 24512-X

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Arnold J. REUSER et al.

Serial No.: 09/886,477

Filing Date: June 22, 2001

For: **METHODS OF PURIFYING HUMAN ACID ALPHA-GLUCOSIDASE**

**Appendix B**

Please cancel presently pending claims 38-39 and amend the following claims as indicated in the following clean copy of the claims.

*Sub 3* 3. (Once Amended) The method of claim 2, wherein the anion exchange column is Q-Sepharose.

*Q 1* 4. (Once Amended) The method of claim 3, wherein the sample is applied to the Q-Sepharose column in low salt buffer and is eluted from the column in an elution buffer of higher salt concentration.

*Sub 5* 5. (Once Amended) The method of claim 2, wherein the anion exchange column is copper chelating Sepharose.

*Q 6* 6. (Once Amended) The method of claim 2, wherein the hydrophobic interaction column is phenyl Sepharose.

8. (Once Amended) The method of claim 2, wherein the hydrophobic interaction column is Source Phenyl 15.

*Q 7* 10. (Once Amended) The method of claim 2, further comprising repeating steps (a) and (b) and/or (c) until the a-

glucosidase has been purified to 95%, preferably 99%, more preferably 99.9% w/w pure.

11. (Once Amended) The method of claim 2, wherein the sample is milk produced by a transgenic mammal expressing the a-glucosidase in its milk.

14. (Once Amended) The method of claim 11, further comprising centrifuging the milk and removing fat leaving skimmed milk.

18. (Once Amended) The method of claim 1, wherein the sample has a volume of at least 100 liters.

22. (Once Amended) At least 95%, preferably at least 99%, more preferably at least 99.9% w/w pure human acid a-glucosidase produced by the process of claim 1.

24. (Once Amended) A pharmaceutical composition comprising human acid a-glucosidase as claimed in claim 19.

25. (Once Amended) Human acid a glucosidase of claim 19 for use as a pharmaceutical.

27. (Once Amended) The use of human acid a-glucosidase of claim 19 for the manufacture of a medicament for treatment of human acid a-glucosidase deficiency.

28. (Once Amended) The use of human acid a-glucosidase of claim 19 for the manufacture of a medicament for intravenous administration for the treatment of human acid a-glucosidase deficiency.

32. (Once Amended) A method as claimed in claim 29 being a batch procedure.

33. (Once Amended) A method as claimed in claim 29, wherein

the hydroxylapatite is in the form of a column, optionally the method is a liquid column chromatography procedure.

34. (Once Amended) A method as claimed in claim 29, wherein the heterologous protein is selected from the group consisting of lactoferrin, transferrin, lactalbumin, coagulation factors such as factor VIII and factor IX, growth hormone,  $\alpha$ -anti-trypsin, plasma proteins such as serum albumin, C1-esterase inhibitor and fibrinogen, collagen, immunoglobulins, tissue plasminogen activator, interferons, interleukins, peptide hormones, and lysosomal proteins such as  $\alpha$ -glucosidase,  $\alpha$ -L-iduronidase, iduronate-sulfate sulfatase, hexosaminidase A and B, ganglioside activator protein, arylsulfatase A and B, iduronate sulfatase, heparan N-sulfatase, galactoceramidase,  $\alpha$ -galactosylceramidase A, sphingomyelinase,  $\alpha$ -fucosidase,  $\alpha$ -mannosidase, aspartylglycosamine amide hydrolase, acid lipase, N-acetyl- $\alpha$ -D-glycosamine-6-sulphate sulfatase,  $\alpha$ -and ss-galactosidase, ss-glucuronidase, ss-mannosidase, ceramidase, galactocerebrosidase,  $\alpha$ -N-acetylgalactosaminidase, and protective protein and others including allelic, cognate or induced variants as well as polypeptide fragments of the same.

35. (Once Amended) A method as claimed in claim 29, wherein the heterologous protein is not one normally found in the milk of an animal.

37. (Once Amended) The method of claim 36, wherein the hydroxylapatite is in the form of a column and the unbound fraction is collected in the flow-through.

**BOX MISSING PARTS**

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A Preliminary Amendment is concurrently filed to removed multiple dependencies from the claims. Examiner is asked to enter this amendment prior to calculating the filing fee.

A Petition for a one month extension of time is submitted with the extension fee of \$55.00 attached to this response.

The Commissioner is hereby authorized to charge any deficiency or credit any excess to Deposit Account No. 14-0112.

Respectfully submitted,

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